

## Reply to Stradner *et al.*: Equilibrium clusters are absent in concentrated lysozyme solutions

In their article (1), Stradner *et al.* stated, “Surprisingly, the peak position . . . is essentially independent” of concentration  $c$  referring to figure 1b in ref. 1 displaying six datasets from  $c = 36$ – $273$  mg/ml. In the main text of our article (2) a clear peak shift in the range of up to  $c = 200$  mg/ml was demonstrated, and supporting information (SI) Fig. S9 (2) specifically presented systematic shifts for six concentrations ( $c = 20$ – $250$  mg/ml). We thus disagree with the claim of “no significant differences” between the data presented in refs. 1 and 2. In their letter (3), Stradner *et al.* partially revoke their original statement (1) to say, “we both observe the usual shift in peak position at low concentrations” and argue about concentrated solutions without quantifying this notion. The peak shift is indeed less pronounced at higher concentrations corresponding to percolating clusters observed in the simulations (4), but this scenario is very different from the compact equilibrium clusters depicted in ref. 1. Our experimental data do not reveal typical small angle scattering features of equilibrium clusters as demonstrated in ref. 2. Instead the data are well described by physically meaningful dimensions and volume fraction of monomers, and this does allow one to reliably distinguish between monomers and clusters. In contrast, simu-

lations of structure factors alone (1, 4) are incomplete because they do not take into account the form factor of the expected clusters but only that of the monomers. Summarizing, we observed no evidence for equilibrium clusters in concentrated lysozyme solutions for conditions similar to those reported in ref. 1. Formation of clusters at yet higher concentrations was neither studied nor questioned in our article (2).

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1. Stradner A, *et al.* (2004) Equilibrium cluster formation in concentrated protein solutions and colloids. *Nature* 432:492–495.
2. Shukla A, Mylonas E, Di Cola E, Finet S, Timmins P, Narayanan T, Svergun DI (2008) Absence of equilibrium cluster phase in concentrated lysozyme solutions. *Proc Natl Acad Sci USA* 105:5075–5080.
3. Stradner A, Cardinaux F, Egelhaaf SU, Schurtenberger P (2008) Do equilibrium clusters exist in concentrated lysozyme solutions? *Proc Natl Acad Sci USA* 105:E75.
4. Cardinaux F, Stradner A, Schurtenberger P, Sciortino F, Zaccarelli E (2007) Modeling equilibrium clusters in lysozyme solutions. *Europhys Lett* 77:48004.

Author contributions: A.S., E.M., E.D.C., S.F., P.T., T.N., and D.I.S. wrote the paper.

The authors declare no conflict of interest.

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